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Purpose: To repeat & optimize preliminarily 6.4 kb.

Tried mini preped DNA as a control

Will try 2 diff. cycling conditions 3 step as well as 2 step.

Colonies will be lysed in 2 different ways 1. in PK (single
2. in H_2O colony
unlysed will also be included again. buffer
↓
Conditions :- since 200 μM dNTP & 2mM pH 7.5, 10 mM Tris. HCl
.4 μM primer has Mg 1 mM EDTA
worked with Taq 5 U, the same 50 μg/ml PK
conditions will be used. ↓

used 2 μl of miniprep - con unknown (used BMB (3).
still have to run agel

Tried diff enzyme con 1, 2, 5 and 1 : .01, 2 : .02, 5 : .05
Taq Taq + DV

Colony lysis: Since these colonies were so small after ON
at 37° pooled 5 or 6 colonies in a single area -
spotted 2 μl of lysis buffer or H_2O mixed & pipetted out the
liquid on to a tube containing 18 μl of lysis buffer
or H_2O

colonies in PK lysis : 55°, 15', → 95°, 10'.

in H_2O : 95°, 15'

(added a 5 μl of H_2O) pooled all three tubes together
and made up the volume to is 50 μl

should have picked more for more reactions
used 10 μl / RX - appropriately either PK lysed or H_2O lysed or
colony itself + 10 μl H_2O

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For miniprep DNA : prepare premix with template

For colony : add to them later

miniprep premix : 25×

A

dNTP	25 µl	(200 µM each / Rx)
F.P	5	(2 pM) 0.4 µM
R.P	5	0.4 µM
Template	25×2	→ last experiment 1.5 + 1 / Rx
miniprep H_2O	415	= 2 µl / Rx.
	500	→ 20 µl / Rx

premix B : 5×

	Taq			Taq + DV		
	1	2	5	1 : .01	2 : .02	5 : .05
(2 pM) Buffer B	50					
dNTP + primer	.5	1	2.5	5	10	25
H_2O	99.5	99	97.5	95	90	75
	150					

→ 30 µl / Rx.

step 3 cycle :

94°, 3'
2× (94°, 45"
55°, 30"
72°, 3')

2 step :

94°, 3'
(94°, 45"
68°, 5') 20

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Tube #	2 step		Tube #	3 step
1	1		13	same as in 2 step.
2	1		14	
3	2		15	
4	2		16	
5	5		17	
6	5		18	
7	1	.01	19	
8	1	.01	20	
9	2	.02	21	
10	2	.02	22	
11	5	.05	23	
12	5	.05	24	

colonies:

Premix A: 15 x

dNTP 15
primer 3
" 3
- 150 (Template 10 µl / Rx to be added later)
H_2O 129

150 → 10 µl / Rx

20 µl → + ← added 30 µl / Rx appropriately either Taq alone / or Taq + DV

Mix B: 5 x as earlier: for T + D.V

for Taq alone used left over from previous page

cycling condition same as minimum prep. for 3 step cycle, 2 step not done ∵ didn't have much template left from lysed plasmid

Tube # 25 1 + .01 }
26 2 + .02 } PK lysed
27 5 + .05 }

28 }
29 } H_2O lysed
30 }

31 }
32 } straight pick
33 }

, 35, 36 (20 Taq)

H₂O: plain

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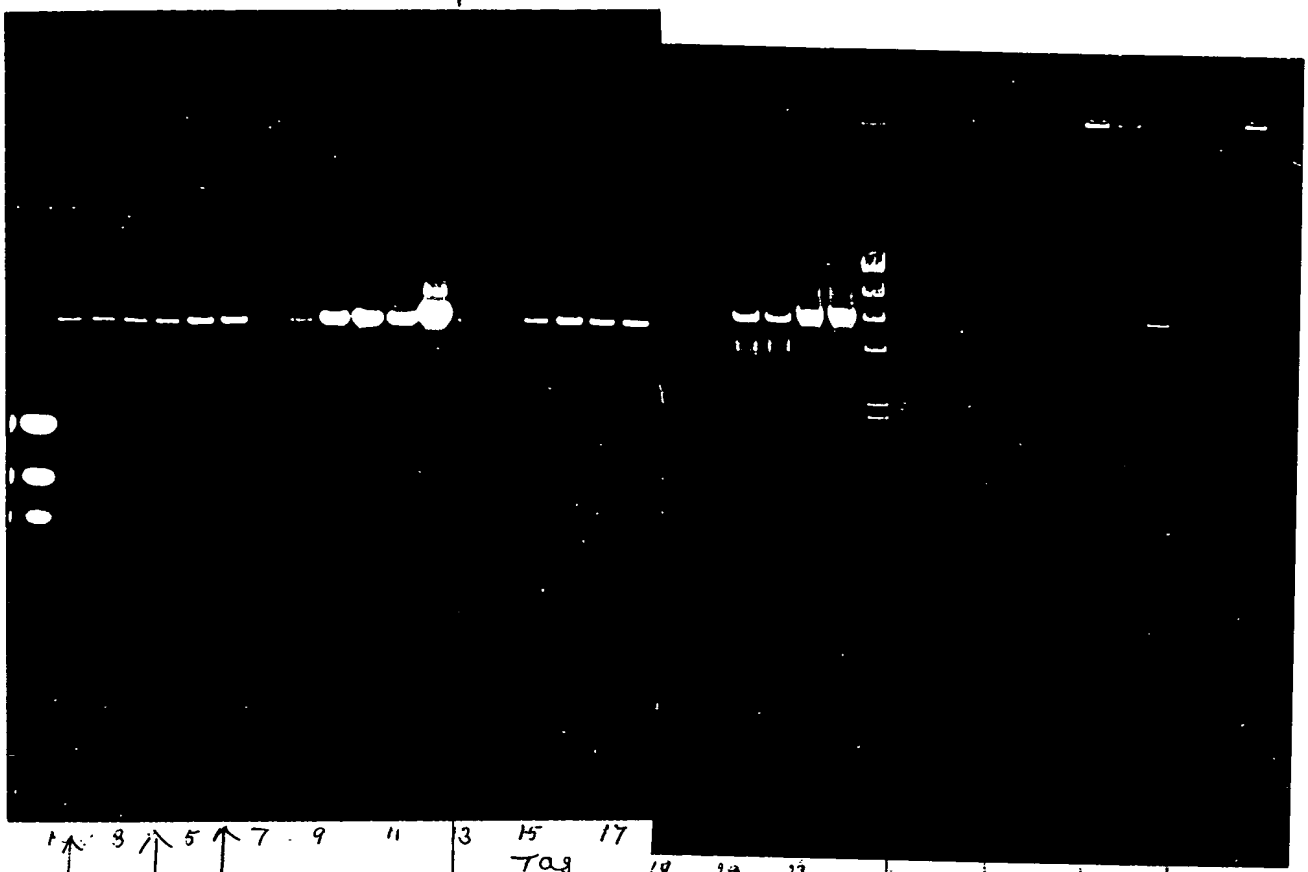

miniprep. plasmids

Result: Even 3 step gave better product with less mis

plasmid amp should be done under more control.

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		Rec rded by K. Haeseman	1/9/95